Induced pluripotent stem cell technology and pancreatic beta cell regeneration

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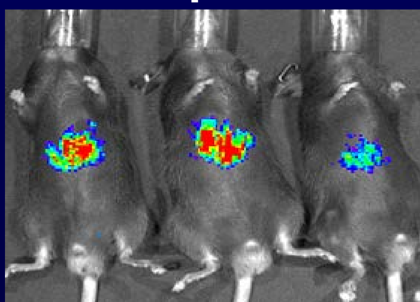
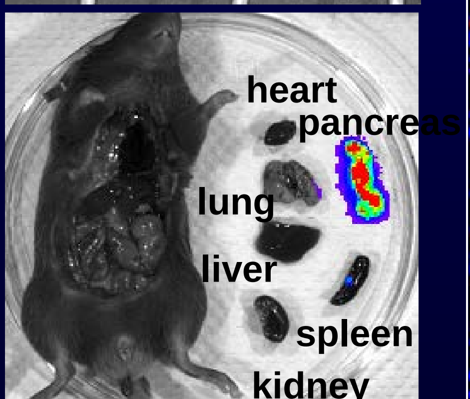
Gene and Cell Therapy for Diabetes and Associated Cardio-Renal Complications

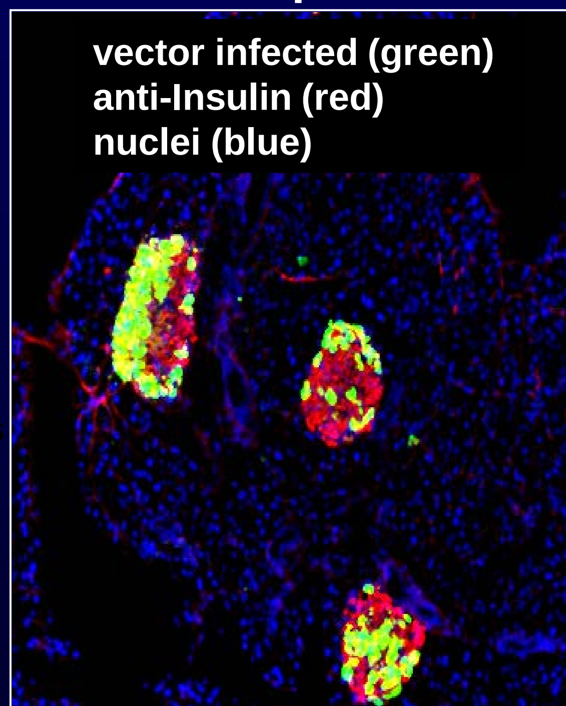
Pancreas-targeting gene transfer vectors for diabetes gene therapy

AAV8 Luc
mINS2 promoter

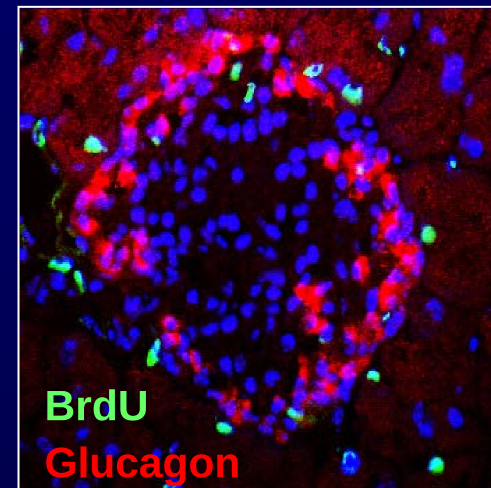




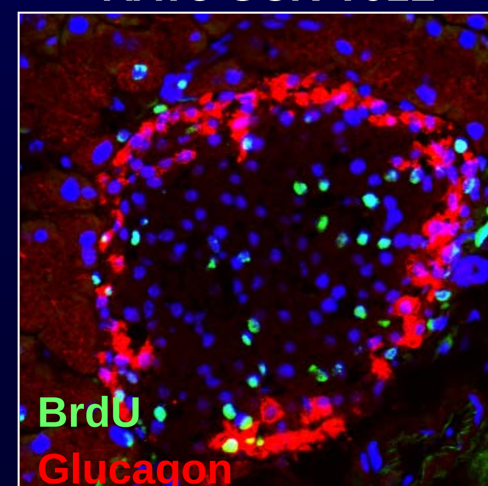
AAV8 GFP
mINS2 promoter



Control



AAV8-GCK V91L



J. Tonne

Outline

1. iPSC technology

2. Intrapatient variations in diabetes-specific iPSC differentiation into insulin-producing cells

3. Regeneration of human islets through in vivo maturation of human iPSC-derived pancreatic endoderm

4. Challenges for future clinical applications

Nuclear reprogramming through induced pluripotent stem cell (iPSC) technology

Yamanaka's group demonstrated that introduction of four pluripotency-associated genes (OCT4, SOX2, KLF4 and c-MYC) reprograms somatic cells into embryonic stem cell-like pluripotent stem cells, called iPSCs.

"2012 Nobel prize in medicine, with Sir. John Gurdon"

Potentials of patient-specific iPSCs

- The iPSC technology enables generation of patient-specific pluripotent stem cells from non-embryonic cell sources.

- Differentiation of patient-derived iPSCs into disease-relevant cell types allows diagnostic and therapeutic applications.

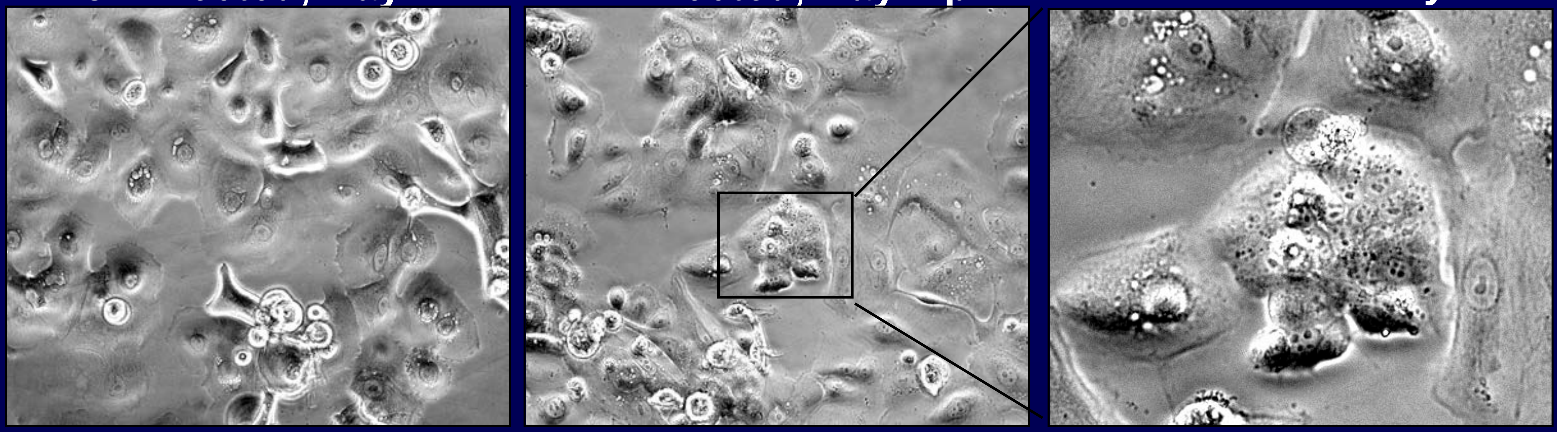
(i) in vitro modeling of patient-specific disease progression

(ii) drug screening

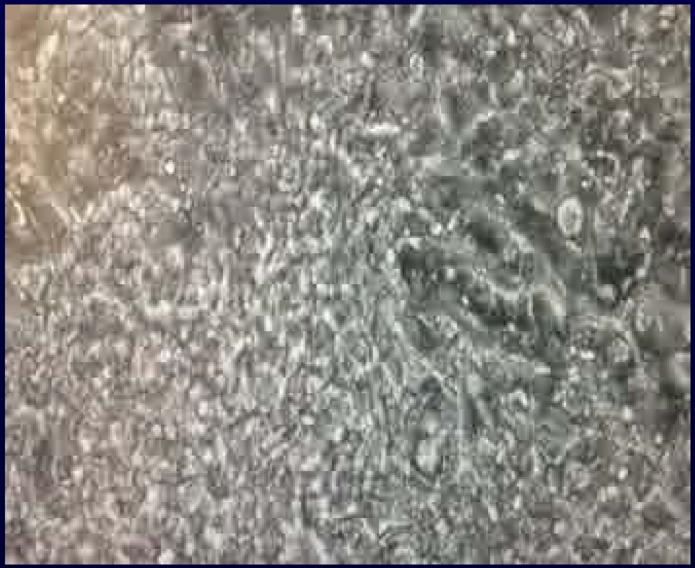
(iii) autologous cell replacement therapies for degenerative disorders

Patient-specific iPSCs

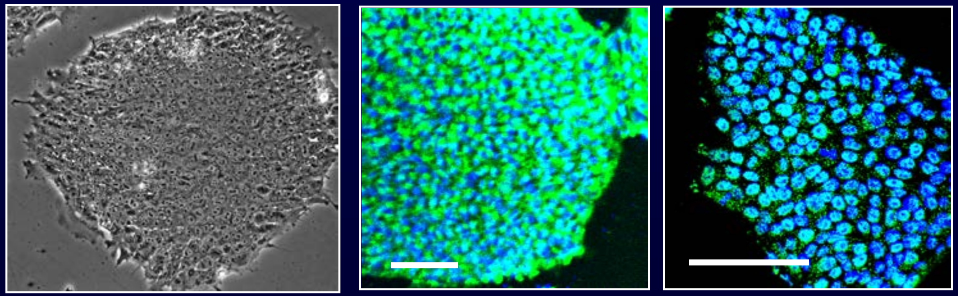
Uninfected, Day 7 **LV-infected, Day 7 p.i.** **iPS-like colony**



iPSC-derived cardiomyocytes



iPSC clone
78Y patient with type 2 diabetes



SSEA4 **NANOG**

Ohmine et al., Aging 2012

Type 1 and Type 2 Diabetes

Both major forms of diabetes involve beta-cell destruction and dysfunction.

Type 1 diabetes is characterized by complete insulin deficiency by autoimmune destruction of islet beta-cells.

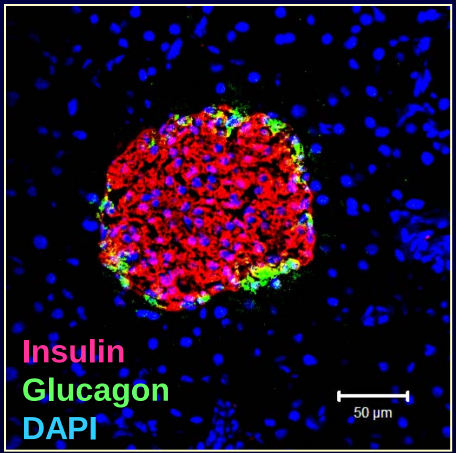
Type 2 diabetes develops when insulin secretory capacity can no longer compensate for peripheral insulin resistance.

Regenerative Medicine for Diabetes

There is great interest in developing strategies to expand the population of functional beta cells.

Possible ways to achieve this include;

- physically replacing the beta cell mass via transplantation
- increasing beta cell replication
- decreasing beta cell death
- deriving new beta cells from appropriate progenitor cells

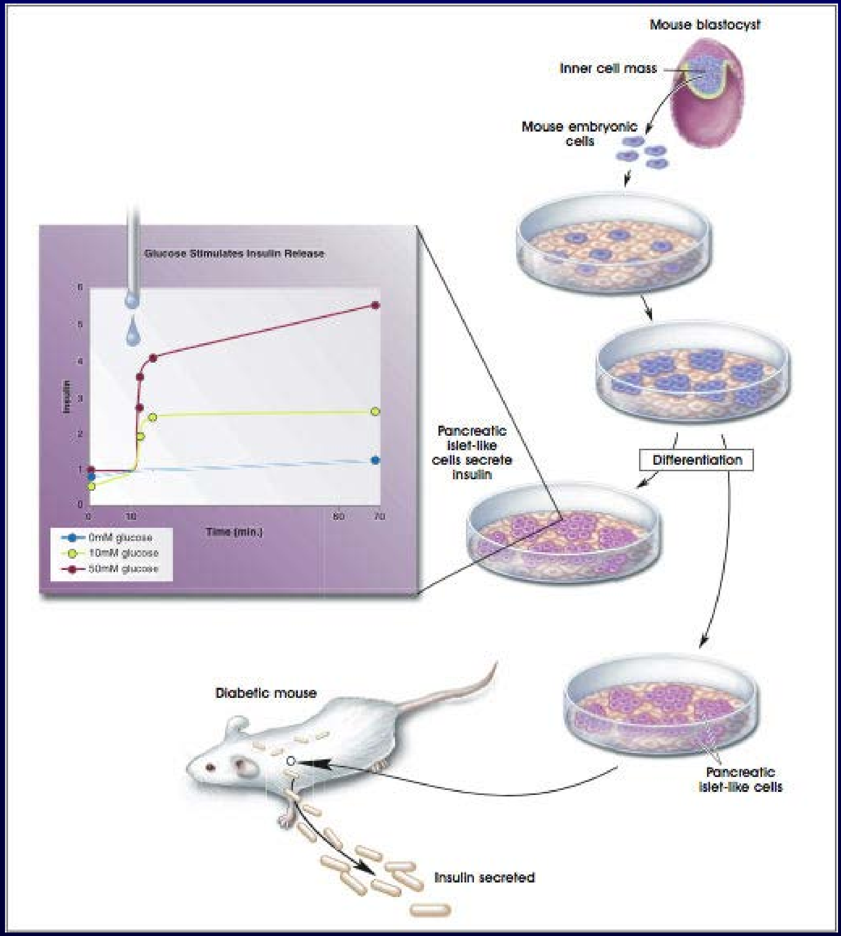


Embryonic stem cells for islet regeneration

Embryonic stem cells can renew themselves infinitely, -- unlimited source for islet regeneration.

Previous studies successfully differentiated human embryonic stem cells into functional islet cells.

However, the use of human embryo-derived cells for therapies is associated with ethical issues and allogeneic mismatch.



http://stemcells.nih.gov/info/scireport/chapter7.asp

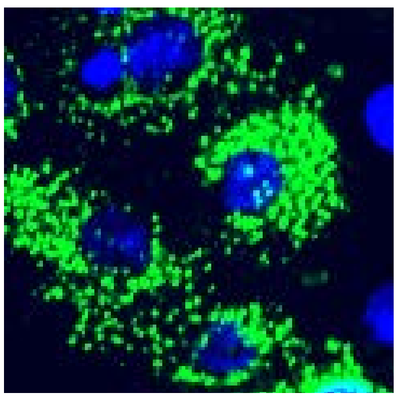
Lumelsky et al., 2001, Science (mouse ES)

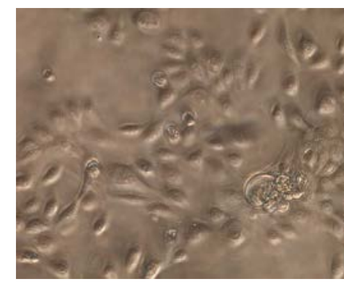
Kroon et al., 2008, Nature Biotechnology (human ES)

MAYO CLINIC

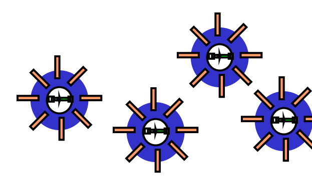
Towards patient-derived iPSCs for a novel cell therapy for type I diabetes

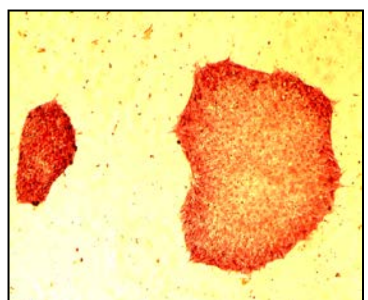
6. Implantation of iPSC-derived islet cells





2. patient cells





5. Generation of islet-like cells

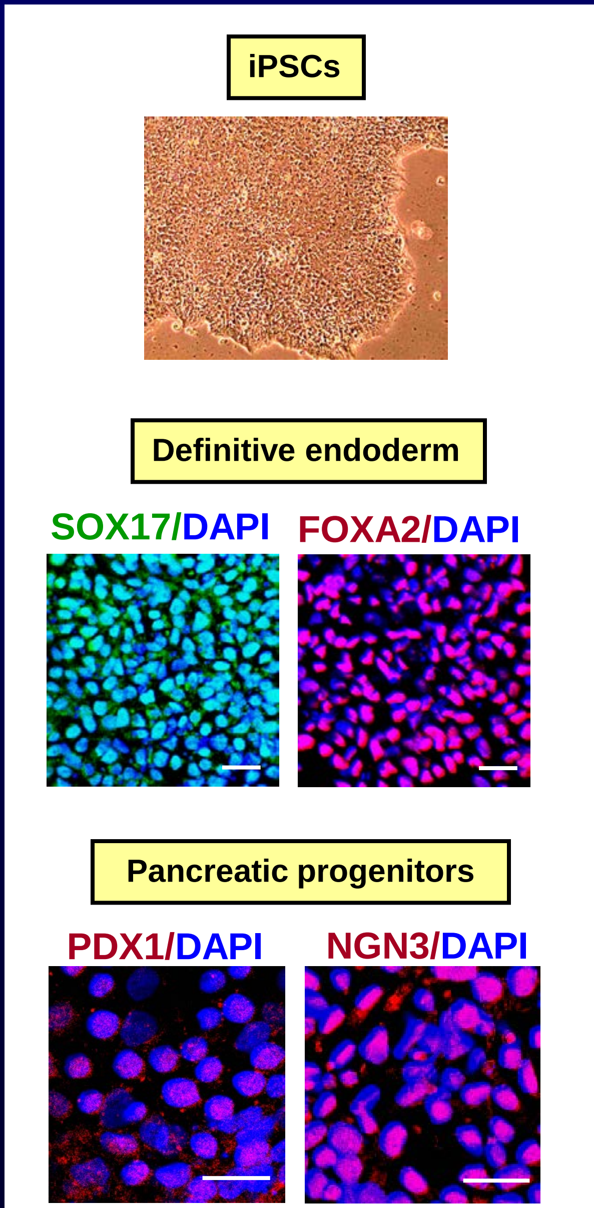
1. iPSC technology

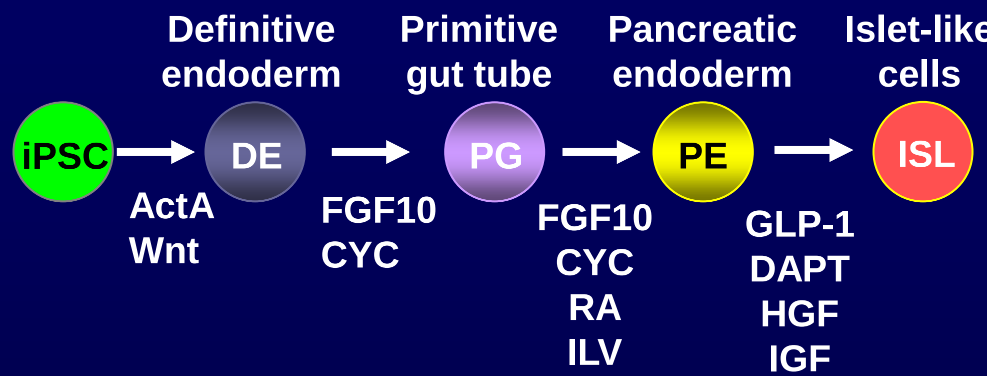
2. Intrapatient variations in diabetes-specific iPSC differentiation into insulin-producing cells

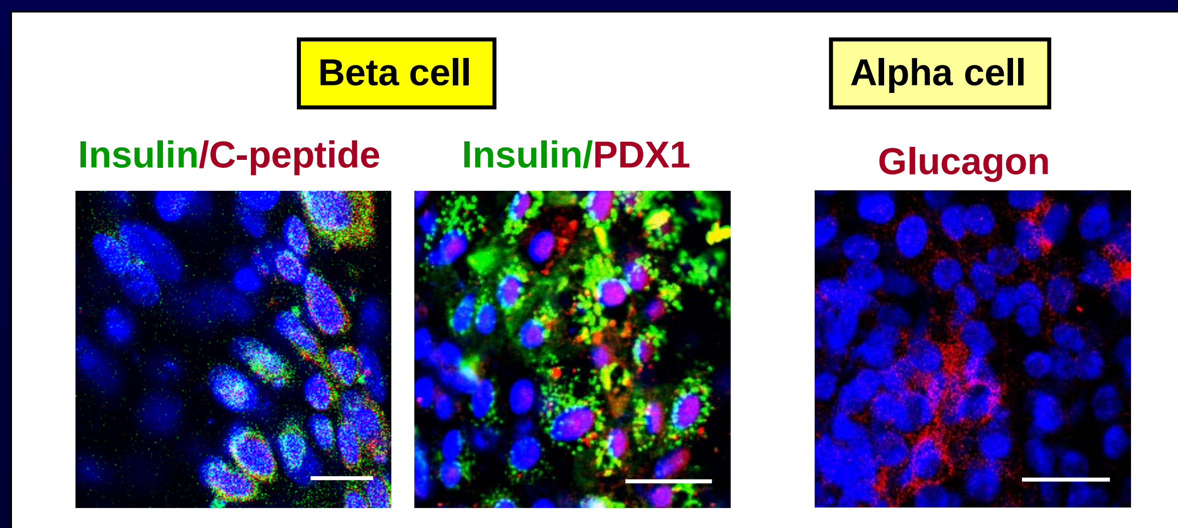
3. Regeneration of human islets through in vivo maturation of human iPSC-derived pancreatic endoderm

4. Challenges for future clinical applications

Guided differentiation of verified iPSCs into insulin-producing cells

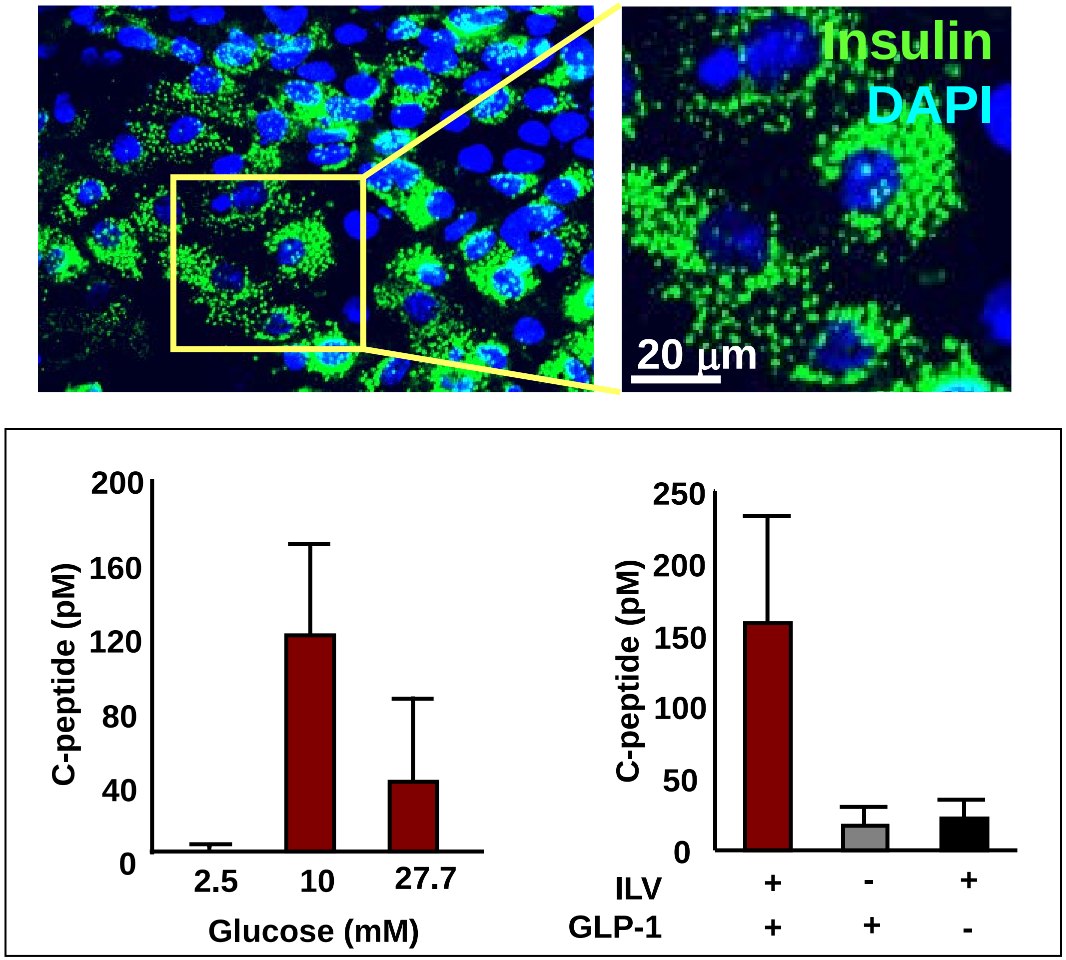






Thatava et al., Gene Therapy 2011

iPSC-derived islet-like insulin-producing cells secrete C-peptide upon Glucose Stimulation



Thatava et al., Gene Therapy 2011

Indolactam V and GLP-1 facilitated generation of glucose-responsive islet like cells

Reproducible differentiation vs. clonal variations

Patient-specific iPSCs can be used for diagnostic and therapeutic applications.

The variations among patient-specific iPSC clones, especially for their pancreatic differentiation propensities, can affect the clinical applications of iPSCs.

To address the influence of the T1D milieu, we recruited T1D patients, generated multiple iPSC clones from each individual, and systematically determined respective differentiation propensities.

Recruited patients with T1D

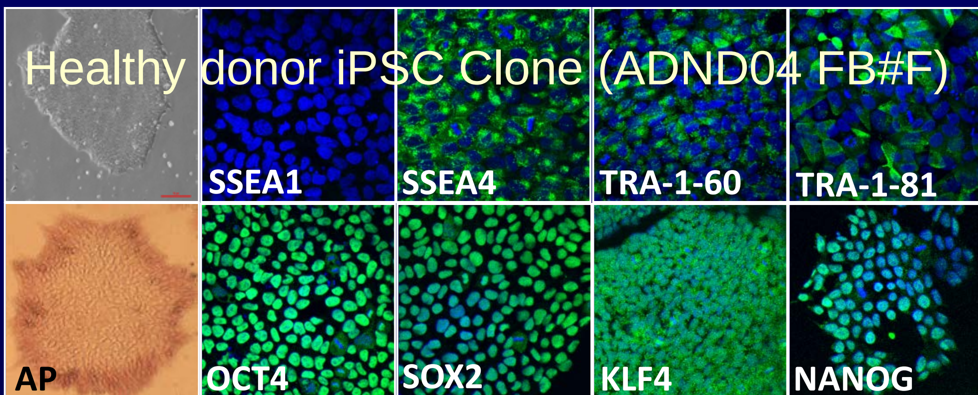
Sample	Age/Sex	Age at diagnosis	Family history of T1D	Medication	Hemoglobin A1c %
ND-1[a]	31/Male	NA	No	NA	NA
T1D-1[b]	38/Female	29	No	Insulin	6.8
T1D-2[c]	47/Male	15	No	Insulin	6.6
T1D-3[d]	21/Male	14	Yes	Insulin	9

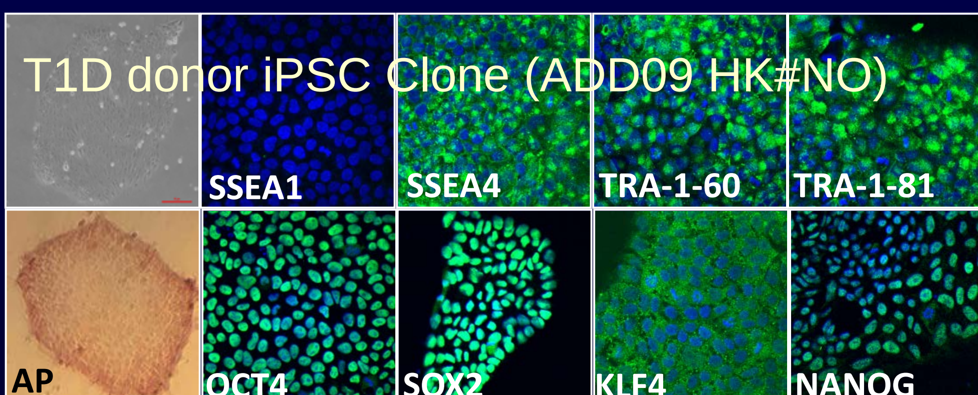
[a] Non-diabetic individual-1; [b] Type 1 diabetic patient-1; [c] Type 1 diabetic patient-2; [d] Type 1 diabetic patient-3. NA – Not applicable

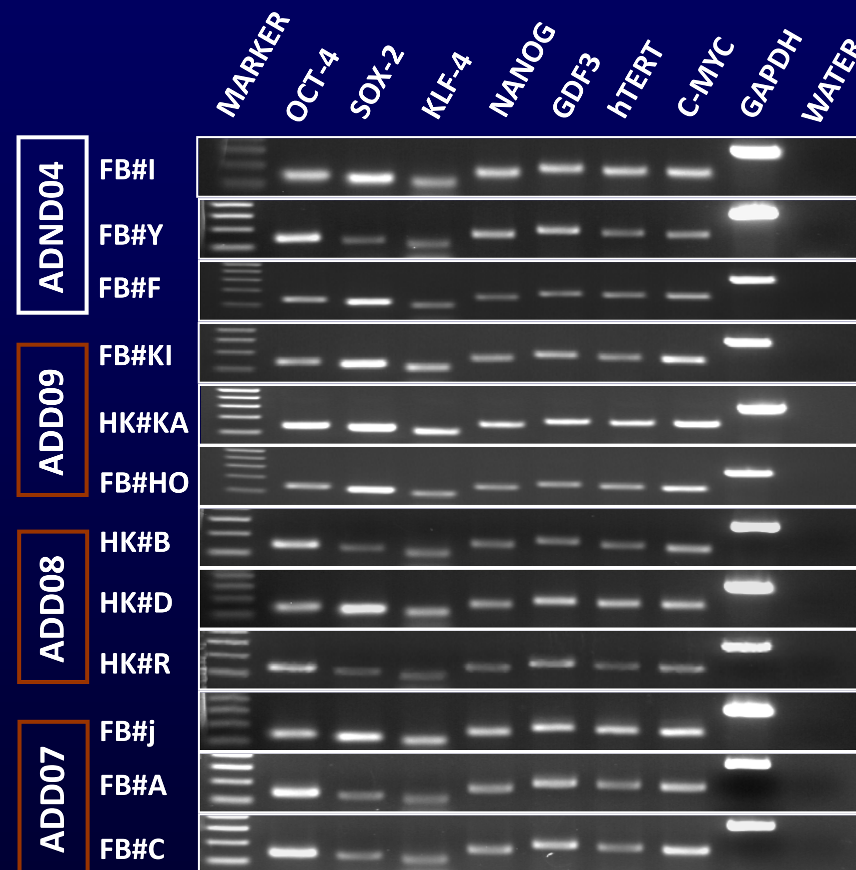
Skin biopsy-derived keratinocytes were reprogrammed by lentiviral vectors expressing OCT4, SOX2, KLF4 and cMYC.

iPSCs were expanded under feeder-free conditions.

Expression of pluripotency markers in patient-specific iPSC clones



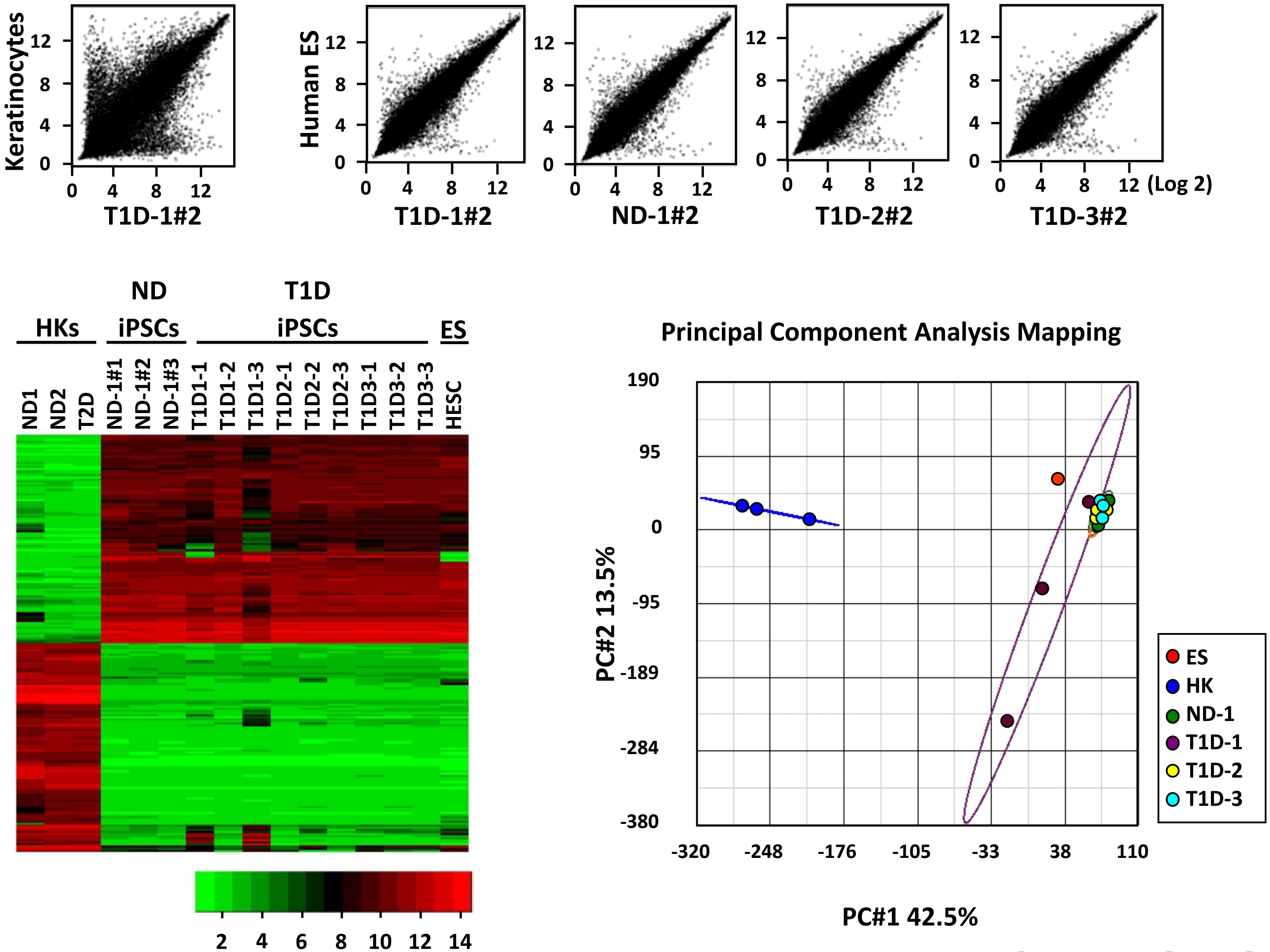




We selected 3 iPSC clones from each donor.

Thatava et al., Mol Ther 2013

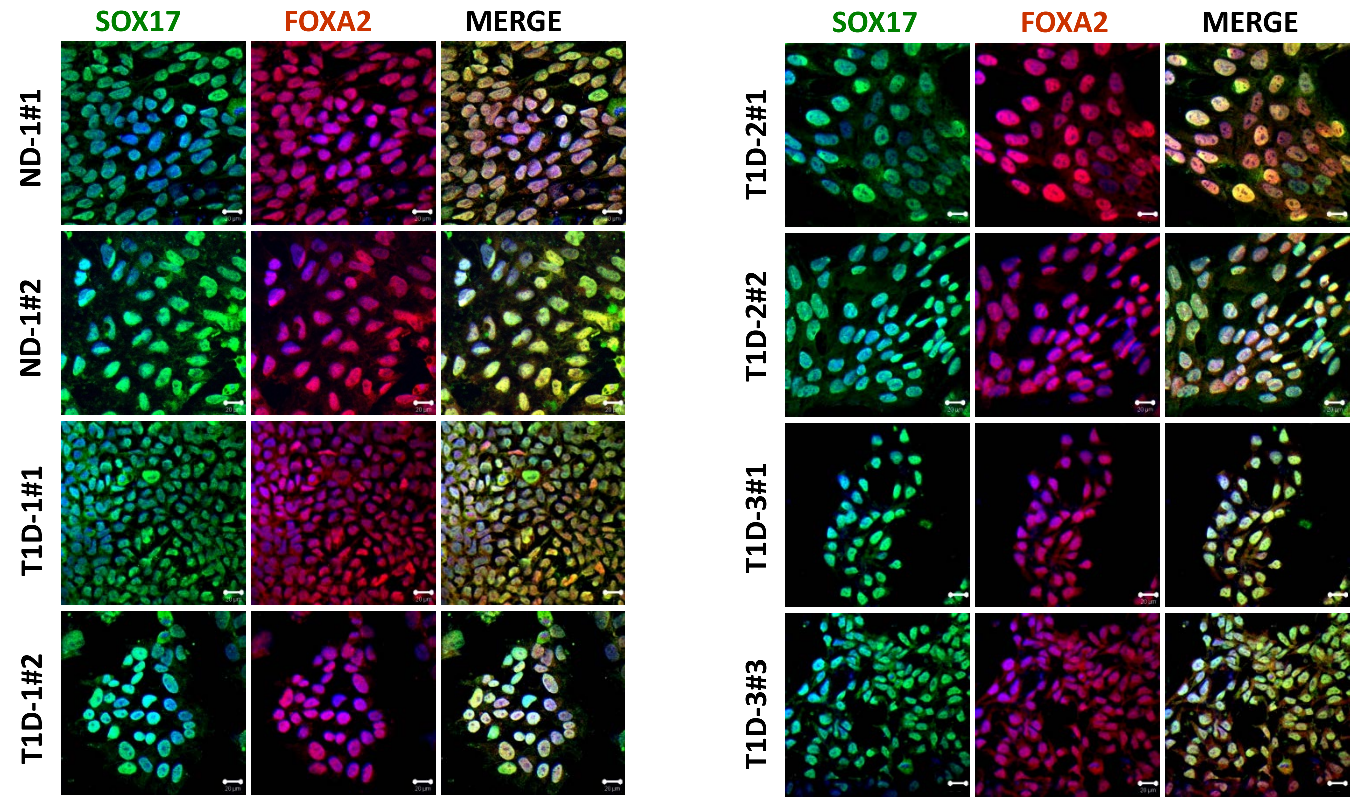
Global gene expression profiles of patient-specific iPSC clones



Thatava et al., Mol Ther 2013

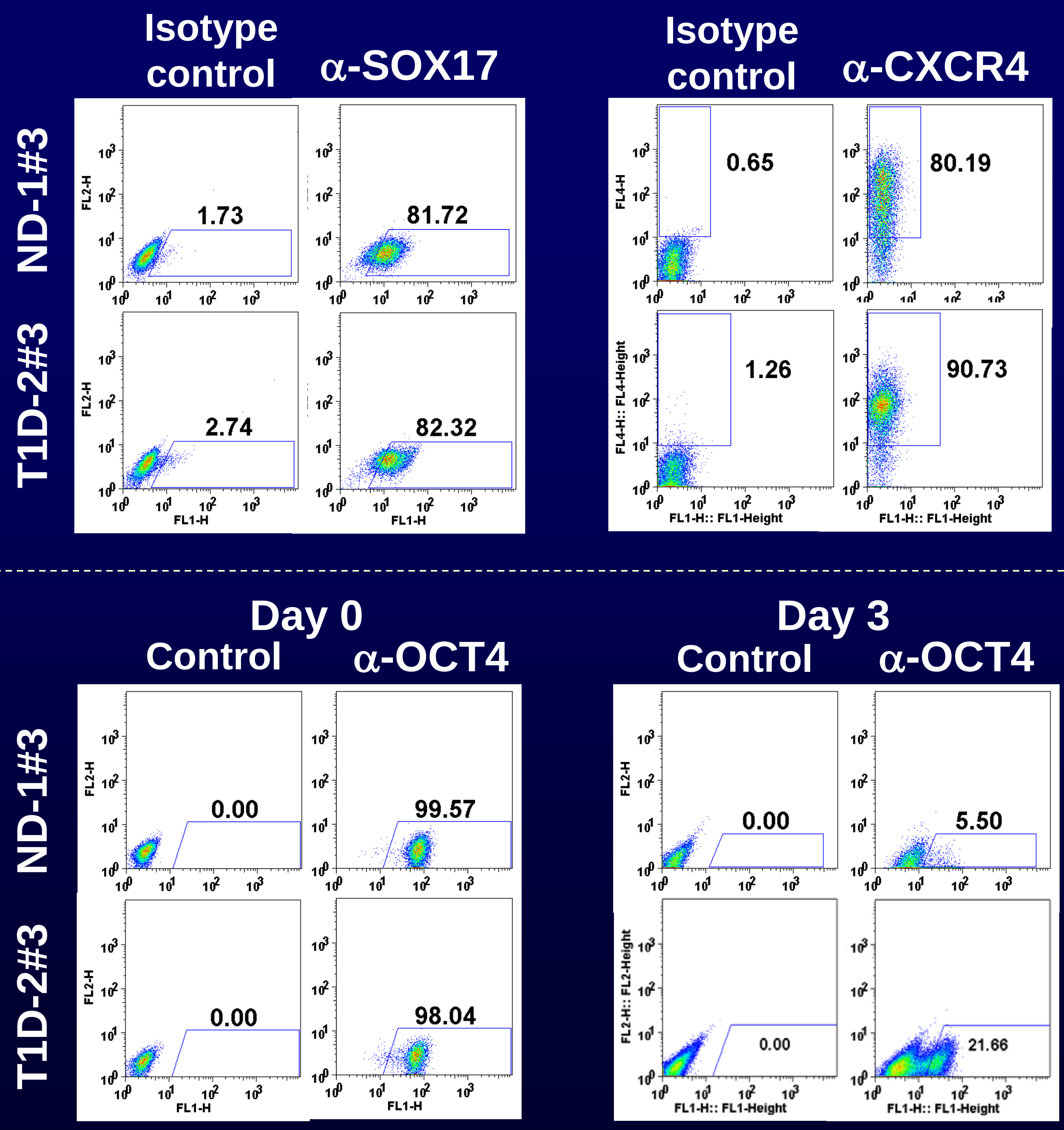
Ten out of 12 T1D-specific iPSC clones showed very similar gene expression profiles, closely related to that of human ESCs.

Efficient differentiation of T1D patient-specific iPSCs into definitive endoderm

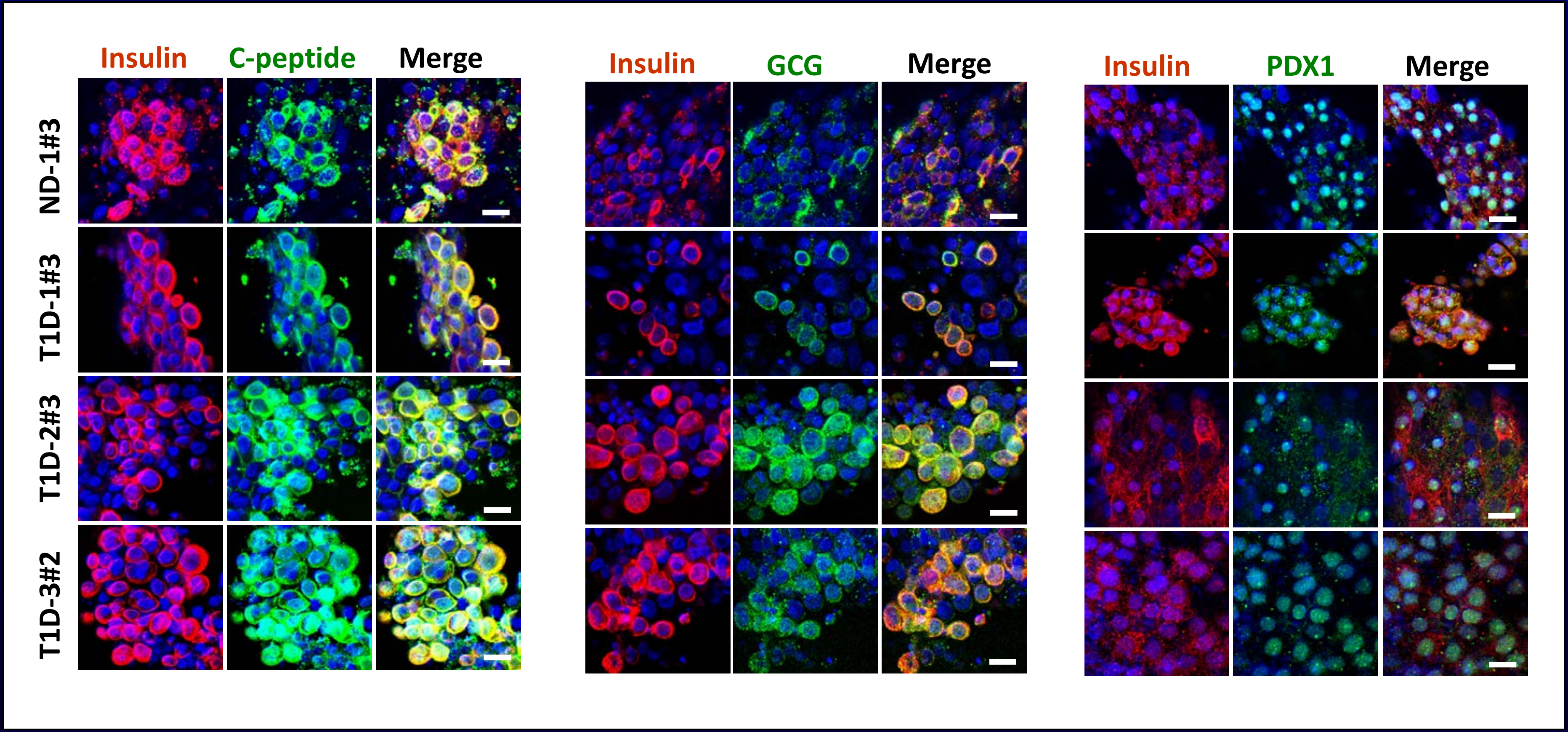


Thatava et al., Mol Ther 2013

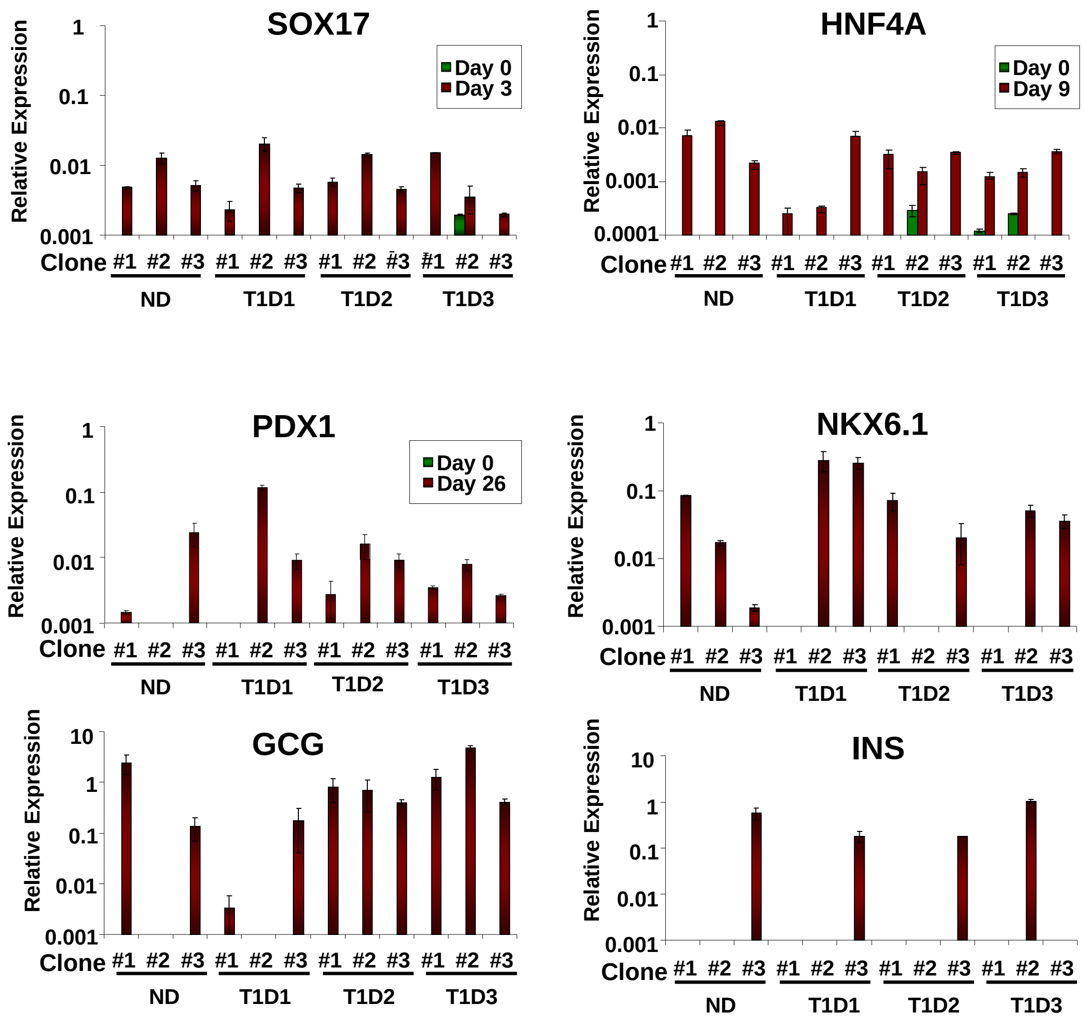
All T1D-specific iPS clones were efficiently differentiated into SOX17 (green)- and FOXA2 (red)-positive endoderm cells.


Isotype control
α-SOX17
Isotype control
α-CXCR4
ND-1#3
T1D-2#3
1.73
81.72
0.65
80.19
2.74
82.32
1.26
90.73
FL2-H
FL1-H
FL4-H
FL4-H:: FL4-Height
FL1-H:: FL1-Height
Day 0
Control
α-OCT4
Day 3
Control
α-OCT4
ND-1#3
T1D-2#3
0.00
99.57
0.00
5.50
0.00
98.04
0.00
21.66
FL2-H:: FL2-Height

Differentiation of T1D-specific iPSCs into endocrine hormone-expressing cells



Only one clone from each donor could be differentiated into insulin-producing cells in vitro.



Day 3 & 9

Day 26

Clonal variations became increasingly prominent upon guided differentiation of iPSC progeny into pancreatic endoderm and islet-like cells.

1. iPSC technology

2. Intrapatient variations in diabetes-specific iPSC differentiation into insulin-producing cells

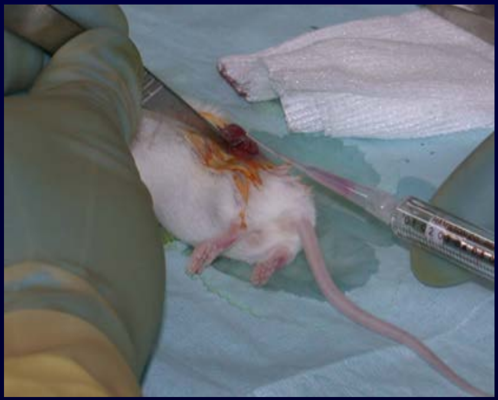
3. Regeneration of human islets through in vivo maturation of human iPSC-derived pancreatic endoderm

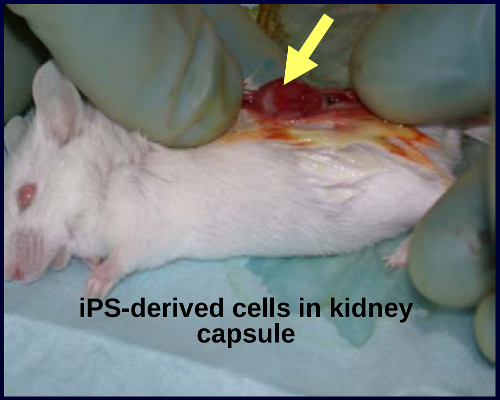
4. Challenges for future clinical applications

In vivo maturation of iPSC-derived pancreatic endoderm cells into functional islets

Transplantation of human ESC-derived pancreatic endoderm cells resulted in generation of glucose-responsive, insulin-producing cells in vivo, which could prevent streptozotocin-induced diabetes in mice (Kroon et al., 2008 *Nat. Biotech*).

We introduced iPSC-derived pancreatic endoderm cells (PDX1-positive cells) in a renal capsule of SCID-beige mice. We used various iPSC clones from different cell sources (skin, blood, heart and stomach) and donors, and over 20 mice were transplanted with the iPSC-derived cells.



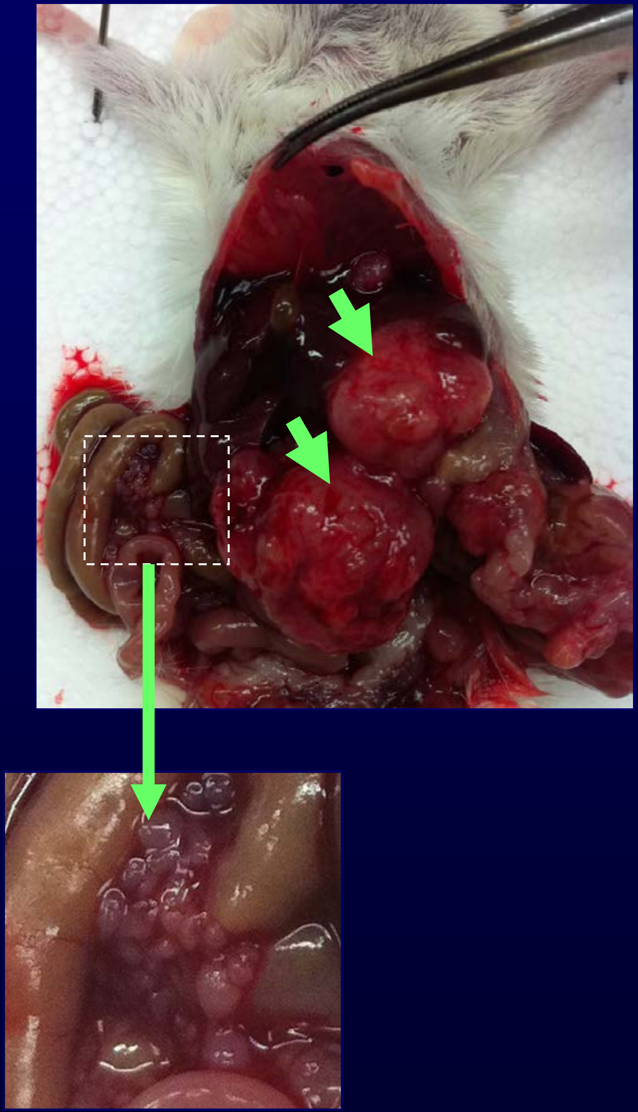


Transplantation of iPSC-derived pancreatic endoderm cells results in teratoma formation

S. Ohmine

Within 6-8 weeks post-transplant, palpable growths were detected in nearly 100% of mice.

Frequent recurrent and metastatic tumors in recipient mice after teratoma removal by nephrectomy



Removal of the iPSC-derived teratoma through nephrectomy frequently led to recurrent tumors.

Some mice showed metastatic tumors in the abdominal cavity, lung or liver, suggesting that iPSC-derived teratoma/tumors are not necessarily benign.

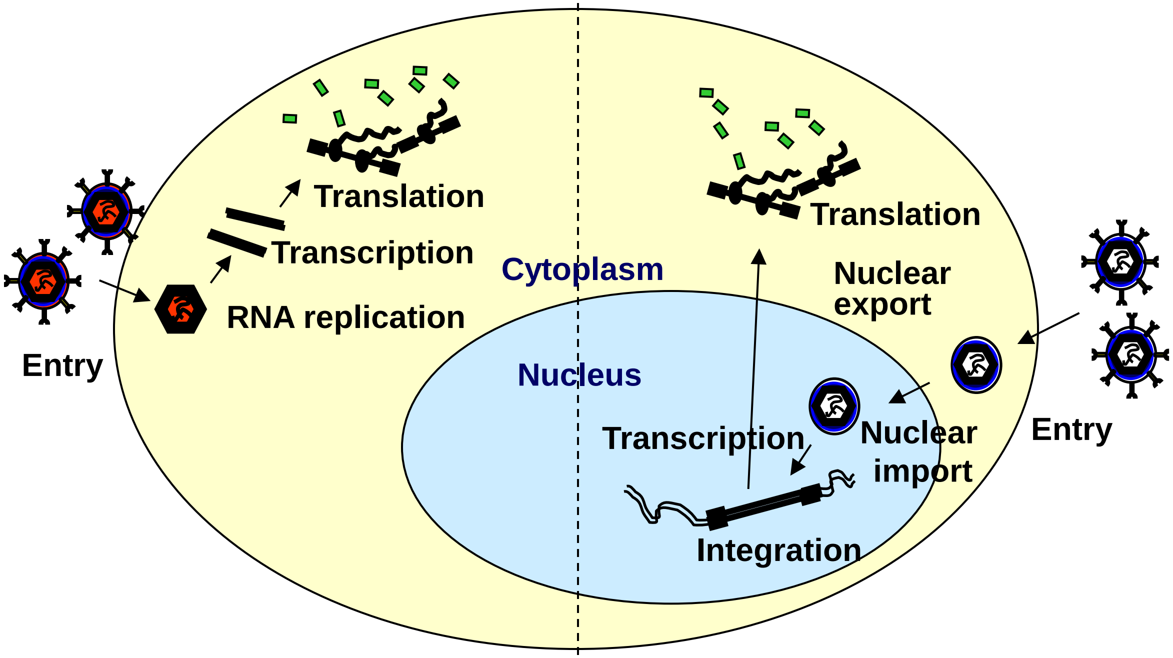
S. Ohmine

- DNA-independent gene expression

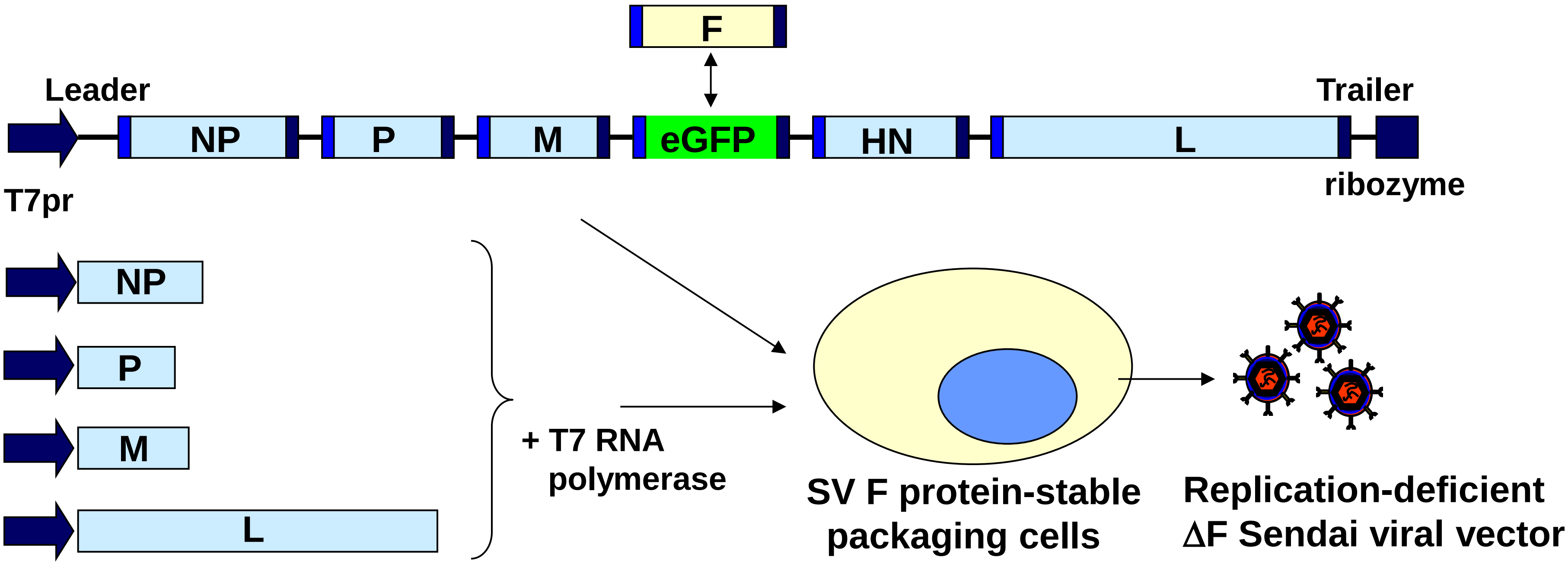
- No integration

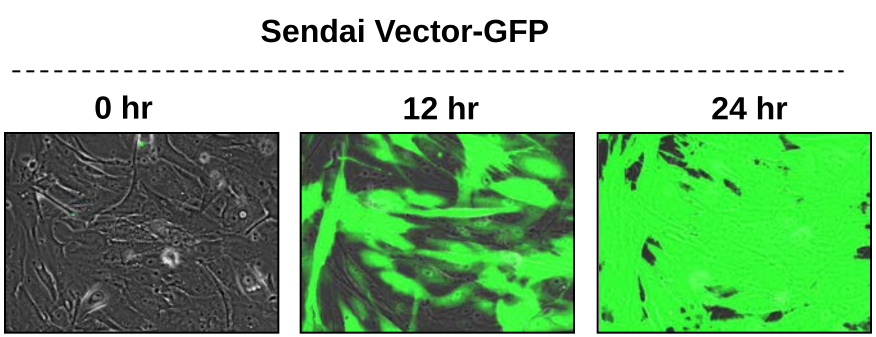
- Vector integration events are associated with the risk of insertional mutagenesis

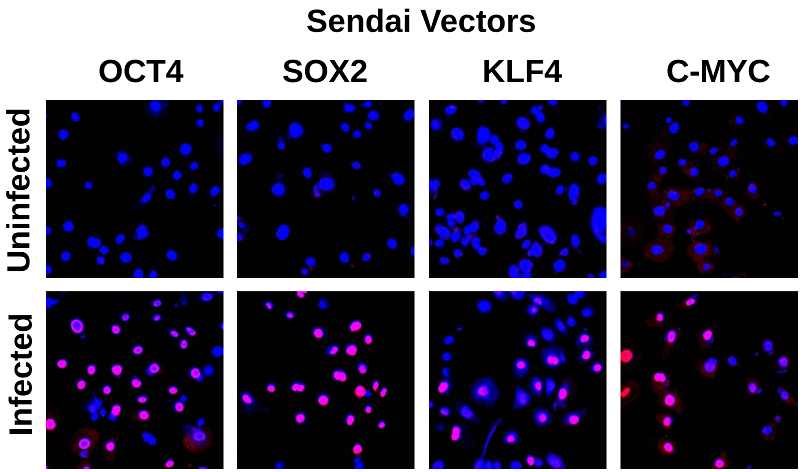
- Sustained expression of c-Myc from integrated vectors can lead to increased tumorigenicity.



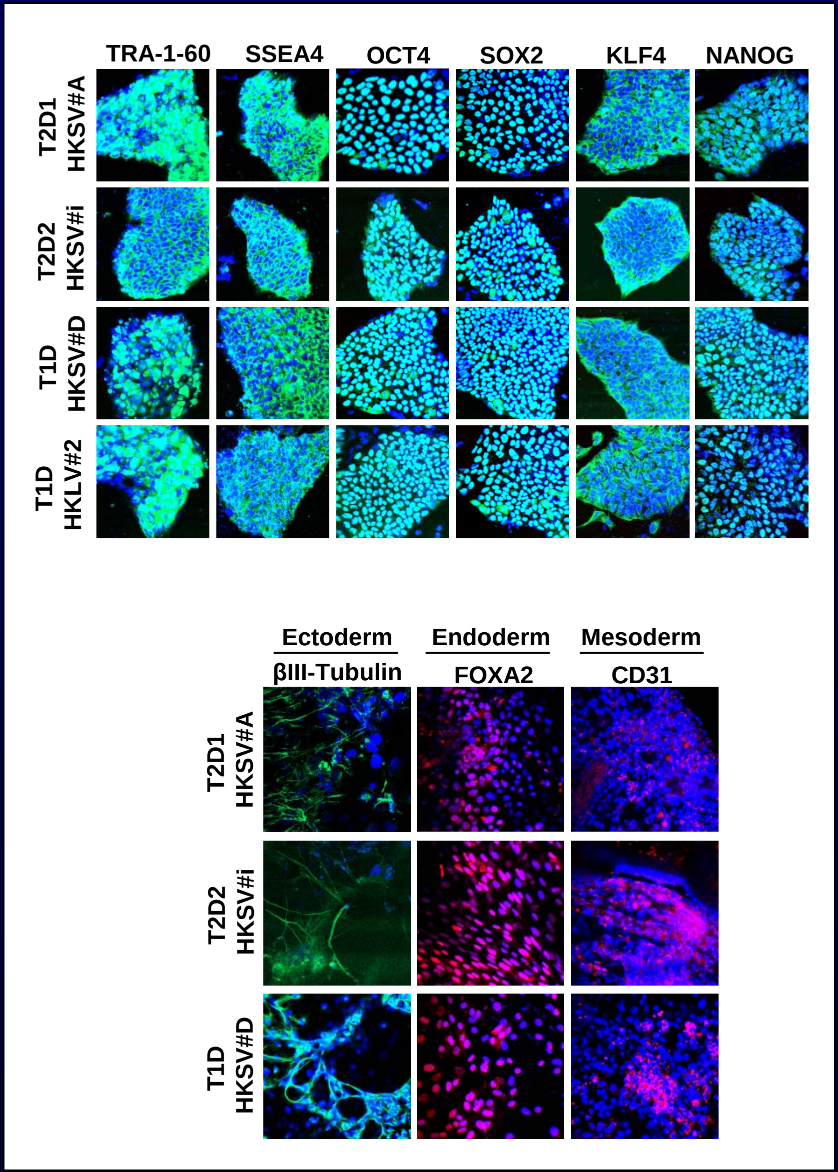
Replication-deficient ΔF Sendai vector (DNAVEC)

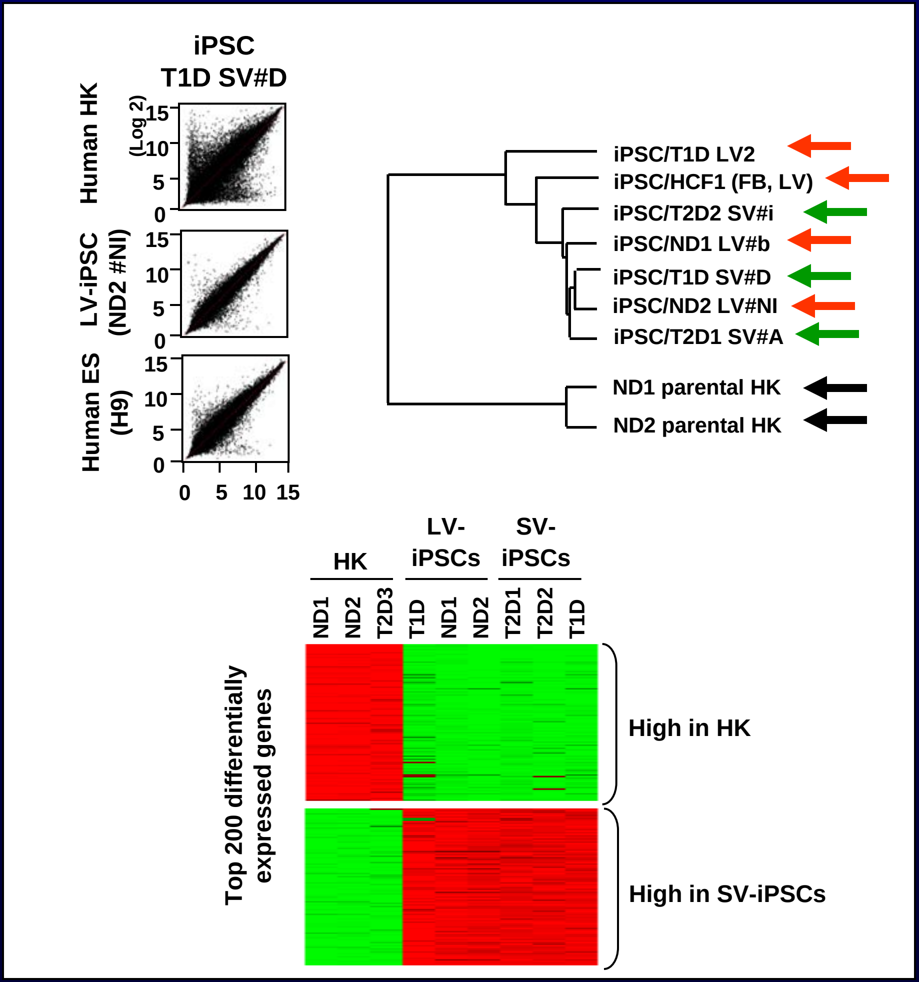






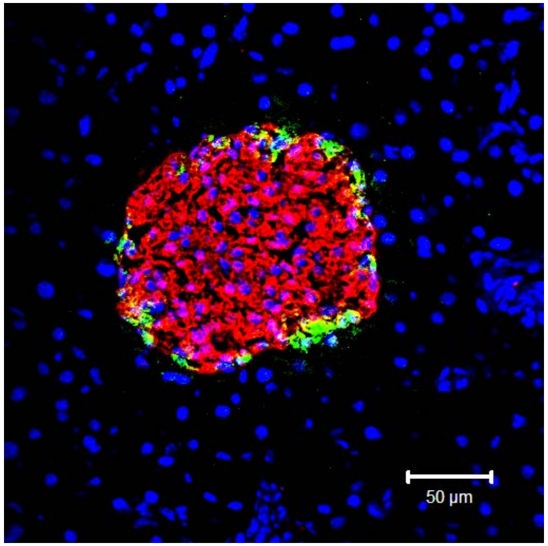
Transgene-free iPS cells from elderly patients with diabetes using non-integrating Sendai virus vectors





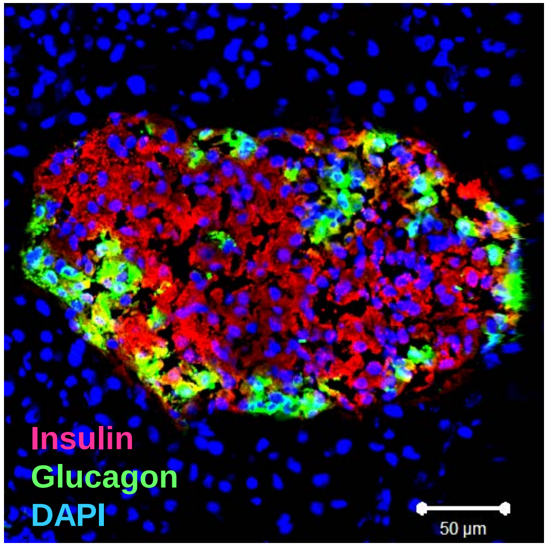
Notable similarities in global gene express profiles between iPSCs made with lentiviral and Sendai vectors.
(*Kudva et al., 2012 Stem Cells Translational Medicine*)

Natural mouse islets

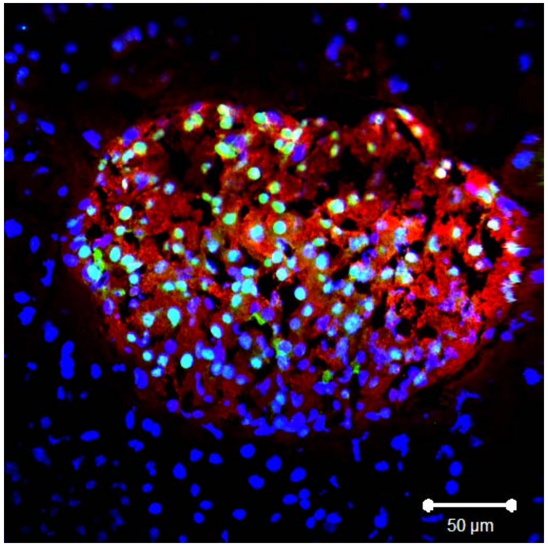


anti-Insulin (red)
anti-Glucagon (green)
nuclei (DAPI, blue)

Transgene-free iPSC-derived islets in kidney capsule (SCID/beige mouse, human blood-derived iPSCs)



anti-Insulin (red)
anti-Glucagon (green)
nuclei (DAPI, blue)



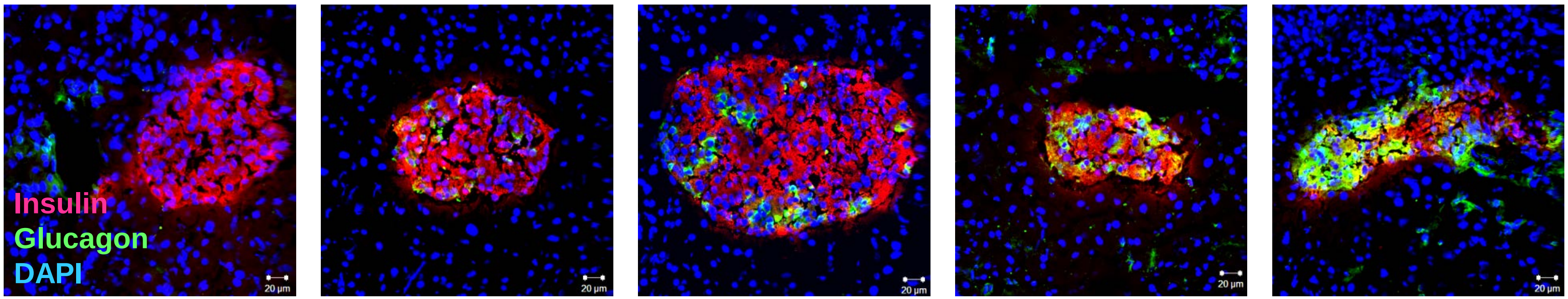
anti-Insulin (red)
anti-PDX1 (green)
nuclei (DAPI, blue)

Similar to human islets, alpha cells were also observed inside of the islets

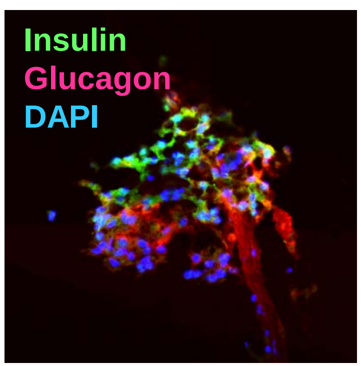
Most beta cells expressed PDX1

S. Ohmine, M. El-Khatib

Variations in composition of cells in iPSC-derived human islets



No alpha cell islet ⟷ **alpha-cell-dominant islet**



Islet made from a type 2 diabetes-patient-derived iPSC clone

We are currently optimizing the conditions for consistent islet regeneration using multiple iPSC clones.

S. Ohmine, M. El-Khatib

Summary

1. iPSCs can be derived from patients with diabetes.

2. Notable intra-patient variation was evident upon further guided differentiation.

3. Transplantation of lenti-iPSC-derived pancreatic endoderm cells resulted in teratoma formation.

4. Use of transgene-free iPSC clones and enzymatic dissociation steps facilitated regeneration of human islets.

- We are currently testing the functionality and therapeutic effects of Sendai-iPSC-derived islets in vivo.

- We are also studying the roles of reprogramming lentiviral vectors on the increased tumorigenicity of iPSC-derived islet-like cells.

- We are going to use T1D patient iPSC-derived islets and autologous immune cells for studying patient-specific immune responses.

Current challenges

Clonal variations among patient-specific iPSCs. – Which clone represents a particular patient?

Relatively low differentiation efficiency

~ 90% induced to definitive endoderm

~ 5% guided to insulin-producing cells

- New promising protocol recently published by Dr. Kieffer's group with human ESCs.

Immature phenotypes of derived islets (limited response to glucose challenge) – Several studies with human ESCs showed generation of mature islet-like cells after 6-8 months of in vivo differentiation.

Further challenges for clinical applications

- Human ESCs were first used in the Geron Spinal Cord Injury Trial (October 2010, discontinued in Nov 2011). Advanced Cell Technology also started two trials with human ESC-derived cells (for Stargardt's Macular Dystrophy and Dry Age-related Macular Degeneration).

- iPSCs = complex cell products (pluripotent stem cells + genetic manipulations). No iPSC trial has been approved by FDA.

- Clinical grade iPSC-islet regeneration requires GMP-grade reprogramming vectors and various cytokines and small molecules for pancreatic differentiation

- Extremely long process for GMP (iPSC derivation/characterization ~1-2 months, pancreatic differentiation for few weeks).

- Sustained autoimmunity in T1D (not in T2D) can reject iPSC-derived islets – encapsulation/immune suppression.

- Balance between risk and benefit. High risk patients with diabetes and end-stage renal disease can generally survive ~10 years with dialysis/insulin treatments. Good safety records required to use iPSC-derived products in diabetes.

Acknowledgments

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Mike Deeds (Islet transplant)
Adam Armstrong (Skin processing)

Slack Lab (University of Minnesota)

Jonathan M. Slack

Dermatology (Skin biopsy)

Josep Genebriera De Lamo
Yulia Krotova Khan

DNAVEC (Sendai Vector)

Noemi Fusaki

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